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Evidence for a Direct Inhibitory Effect of PYY on Insulin Secretion in Rats

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Summary: Peptide YY (PYY) has been shown to inhibit stimulated insulin secretion under in vivo conditions in the mouse, the rat, and the dog. In the present study, we investigated the effects of PYY on insulin secretion from the isolated perfused rat pancreas and isolated rat islets. In isolated pancreas perfused in presence of 8.3 m*M* glucose, PYY at 10^{-10} and 10^{-9} *M*, but not at 10^{-8} *M*, inhibited insulin secretion. In the presence of 5.5 m*M* glucose, PYY (10^{-9} *M*) did not modify basal insulin release but reduced the biphasic insulin response to arginine (10 m*M*). PYY also markedly reduced the pancreatic vascular flow rate; this effect was observed at all three concentrations tested in a dose-dependent manner. In isolated islets, glucose (15 m*M*)–stimulated insulin secretion was inhibited by PYY at 10^{-7} *M*. We conclude that in the perfused rat pancreas, PYY inhibits insulin secretion and induces vasoconstriction without a causal relationship. In addition, our results on isolated islets suggest that the inhibitory action of PYY on insulin secretion is exerted through a direct islet action. **Key Words:** Insulin secretion—Islet—Pancreas—Peptide Y.

Peptide YY (PYY) is an amidated peptide consisting of a 36–amino acid residue, originally isolated from the porcine intestine (1). The peptide shows a high degree of structural similarity with pancreatic polypeptide and neuropeptide Y (2). Furthermore, PYY has been demonstrated to be localized both in intestinal cells and in pancreatic A-cells (3–5). Moreover, electron microscopical immunocytochemistry has revealed that PYY and glucagon are co-stored in the secretory granules of pancreatic A-cells (5). This suggests that PYY might be an intraislet regulatory peptide. In addition, PYY might be a gut hormone of importance after feeding, since its plasma concentration is elevated postprandially (6).

PYY has been demonstrated to inhibit stimulated insulin secretion under in vivo conditions in the rat (7), the dog (8,9), and the mouse (5). The aim of the present study was to investigate, in the perfused rat pancreas, whether PYY exerts this effect through a direct pancreatic action on insulin secretion. Furthermore, since PYY markedly reduced pancreatic vascular flow rate, experiments were also performed on isolated islets to ascertain a direct islet effect of PYY.

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MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 330–350 g, were used. The rats were fed a standard pellet diet ad libitum.

Exhibit 5

Experiments on perfused pancreas

The surgical procedure for the isolated perfused rat pancreas has been described previously (10,11). After anesthesia with sodium pentobarbitone (60 mg/kg i.p.), the pancreas was totally isolated from all neighboring tissues. The pancreas was perfused through its arterial system with a Krebs-Ringer bicarbonate buffer containing 2 g/L pure bovine serum albumin (BSA). The Krebs buffer had the following ionic composition (in m*M*): NaCl 108, KH_2PO_4 1.19, KCl 4.74, $CaCl_2$ 2.54, $MgSO_4 \cdot 7H_2O$ 1.19, and $NaHCO_3$ 18. A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was 7.4. The preparation was maintained at 37.5°C. The circulation of the perfusion fluid was performed with a peristaltic pump, ensuring a constant output. The perfusion pressure, measured with a water manometer, was constant and imposed by a pressure limiter, which allowed evacuation of a part of the liquid pulsed by the pump. This liquid, sent back to the origin reservoir, represents the difference between the pump output and the flow rate accepted by the pancreas at the imposed pressure. So, any change in pancreatic vascular bed resistance was detected by measuring pancreatic effluent output. The pressure (ranging between 35 and 45 cm H_2O) was selected to give a flow rate of ~2.5 ml/min during the stabilization period.

In all experiments, a 30-min adaptation period was allowed before taking the first sample. Then two more samples were taken at 40 and 45 min, the latter representing the reference sample. The Krebs solution supplemented with synthetic porcine PYY (Sigma Chemical Co., St. Louis, MO, U.S.A.) was then perfused for 30 or 40 min. The flow rate was measured during 1 min for each sample, which was then immediately frozen for insulin and glucagon radioimmunoassays.

Experiments on isolated islets

Islets were isolated after collagenase digestion of the pancreas according to the technique of Lacy and Kostianovsky (12). Immediately after isolation,

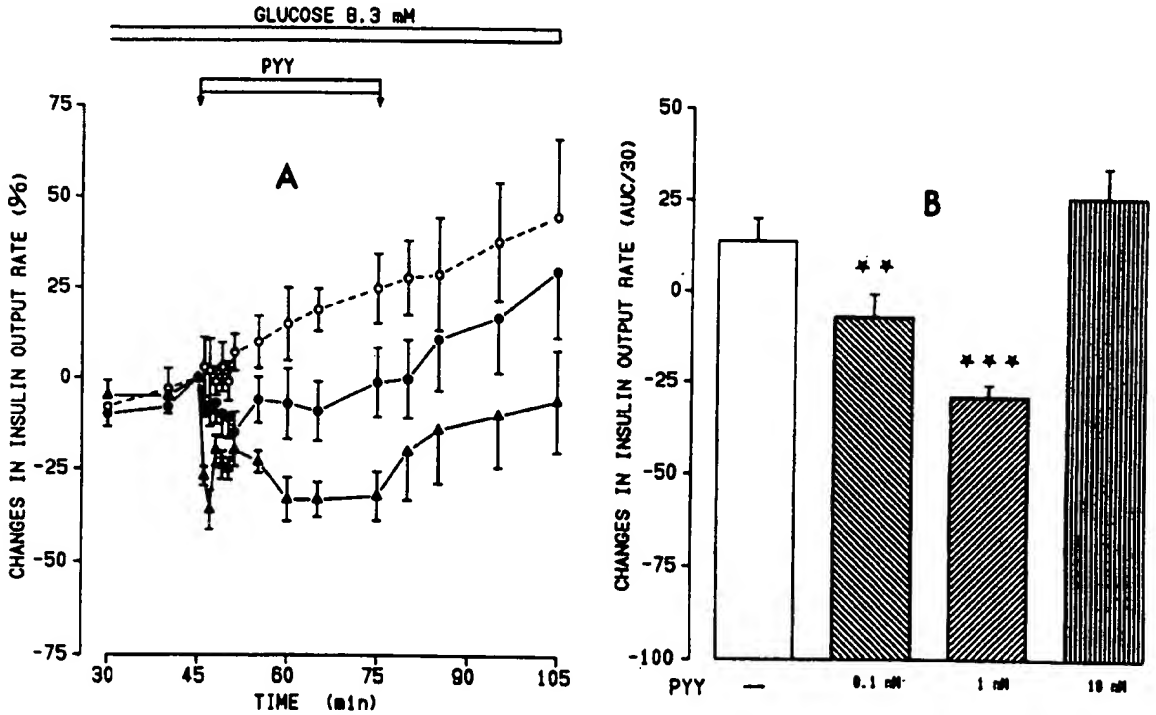

FIG. 1. Effects of PYY on glucose (8.3 m*M*)-induced insulin secretion from isolated perfused pancreas of the rat. A: PYY at 10^{-10} *M* (●) and 10^{-9} *M* (▲); controls (○). B: Integrated insulin responses (AUC/30). The insulin output rates (ng/min) at 45 min were 18.5 ± 2.8, 20.9 ± 2.9, and 21.2 ± 1.0 for PYY at 10^{-10}, 10^{-9}, and 10^{-8} *M*, respectively, and 22.7 ± 1.3 for controls. Values are means ± SEM of five experiments. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. controls.

the islets were preincubated for 90 min at 37.5°C in a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1 g/L BSA and 15 m*M* glucose. Thereafter, batches of three islets were incubated for 60 min in 1 ml of medium containing appropriate concentrations of glucose and PYY. A fraction of the medium was taken at the end of the incubation for insulin assay.

Insulin and glucagon assays

Insulin was assayed by the method of Herbert et al. (13) using the antibody supplied by Miles Laboratories (Paris). ^{125}I-Insulin was obtained from international CIS (Gif-Sur-Yvette, France). Pure rat insulin (Novo, Copenhagen, Denmark) was used as the reference standard, the biological activity of which was 22.3 μU/ng. The intra- and interassay variations were, respectively, 9 and 13.5%.

Glucagon concentrations were measured by the method of Unger et al. (14) using the BR 124 glucagon antiserum from the Institut de Biochimie Clinique (Centre Médical Universitaire, Geneva, Switzerland). The intra- and interassay variations were 10 and 15%, respectively.

Expression of data and statistical analysis

For the kinetics of insulin output rate and flow rate, the results are expressed as changes in relation to the value at time 45 min taken as 100%. The average changes in insulin output rate and flow rate were calculated by dividing the area under the curve (AUC) during the 30-min infusion by 30. Data are expressed as means ± SEM and were submitted to analysis of variance followed by the multiple comparison test (15).

RESULTS

Effects of PYY in isolated perfused pancreas

Effects on insulin and glucagon secretions

In the presence of 8.3 m*M* glucose, PYY at 10^{-10} and 10^{-9} *M* caused a sustained reduction of insulin secretion that was dose dependent (Fig. 1A). Thus, the mean decrease in insulin output rate induced by PYY at 10^{-10} *M* was −7 ± 6% ($p < 0.01$) vs. +14 ± 6% in controls; and with PYY at 10^{-9} *M*, the reduction was −29 ± 3% ($p < 0.001$) (Fig. 1B). At 10^{-9} *M* PYY, the inhibition of insulin release displayed a biphasic pattern. In contrast, at the higher concentration of 10^{-8} *M* (Fig. 2), PYY elicited a decrease in insulin output rate only during the first minute (−53 ± 5%), and thereafter no inhibitory

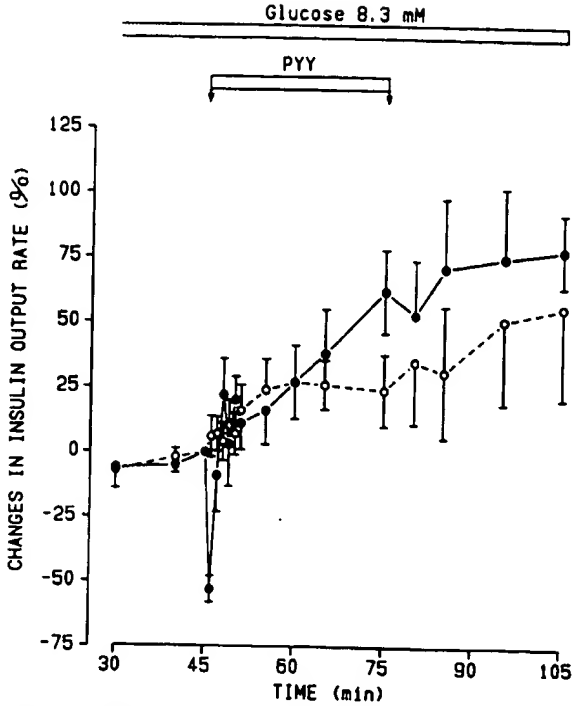

FIG. 2. Effect of PYY at 10^{-8} *M* (●) on glucose (8.3 m*M*)–induced insulin secretion from isolated perfused pancreas of the rat. Controls (○). The insulin output rate (ng/min) at 45 min was 17.4 ± 6.8 for PYY and 24.3 ± 6.3 for controls. Values are means ± SEM of six experiments.

effect was noted; the mean insulin output rate was +26 ± 8% (NS vs. controls; Fig. 1B).

The effect of PYY on glucagon output is shown in Table 1. At 10^{-9} *M*, PYY did not significantly modify glucagon output rate. At 10^{-8} *M*, PYY induced only a transient reduction during the first 3 min ($p < 0.05$).

In the presence of 5.5 m*M* glucose (Fig. 3), PYY at 10^{-9} *M* did not significantly affect basal insulin and glucagon output rates. When insulin and glucagon secretions were stimulated by L-arginine infusion (10 m*M*), PYY (10^{-9} *M*) reduced the biphasic insulin response to the amino acid ($p < 0.05$) (Fig. 3A) but did not affect glucagon response (Fig. 3B).

Effects on vascular flow rate

At the three concentrations tested, PYY provoked a rapid and sustained decrease of pancreatic flow rate. This effect occurred in a dose-dependent manner. At 10^{-10} *M*, the reduction was rapid and remained stable at −12%. At 10^{-9} *M*, the reduction was more pronounced and occurred in a biphasic

TABLE 1. *Effects on glucagon output rate of a 30 min. infusion of PYY at 10^{-9} and 10^{-8} M in isolated pancreas of rat perfused in presence of 8.3 mM glucose*

	Time (min)														
	30	40	45	46	47	48	49	50	55	60	65	75	85	95	105
Controls	119 ±22	112 ±16	100	92 ±9	93 ±10	95 ±14	101 ±14	104 ±15	98 ±24	102 ±23	103 ±20	127 ±19	130 ±35	155 ±39	200 ±31
PYY 10^{-9} *M*	108 ±10	104 ±6	100	102 ±11	76 ±10	85 ±14	107 ±23	108 ±27	134 ±30	151 ±46	176 ±60	169 ±57	177 ±71	146 ±47	156 ±63
PYY 10^{-8} *M*	97 ±10	107 ±6	100	54[a] ±9	45[a] ±3	49[a] ±6	55 ±6	71 ±5	78 ±9	87 ±12	101 ±18	152 ±52	248 ±97	243 ±78	213 ±45

Values are means ± SEM of four to six experiments. Glucagon output rates (pg/min^{-1}) at 45 min were 283 ± 63 and 215 ± 60 for PYY at 10^{-9} and 10^{-8} *M*, respectively, and 206 ± 28 for controls.
[a] $p < 0.05$.

pattern (Fig. 4A). The same pattern was obtained with 10^{-8} *M*. The maximum decrease (−49 and −65%, respectively, for 10^{-9} and 10^{-8} *M*) appeared during the first 2 min; thereafter, the flow rate returned to higher values (−34% and −39% from 10 min).

Figure 4B shows the mean decreases per minute in pancreatic flow rate for the three concentrations of PYY tested.

Effects on isolated islets

Basal insulin release recorded in the presence of low glucose (3 m*M*) was 0.45 ± 0.04 ng/h/islet (Table 2). Glucose at 15 m*M* elicited a significant stim-

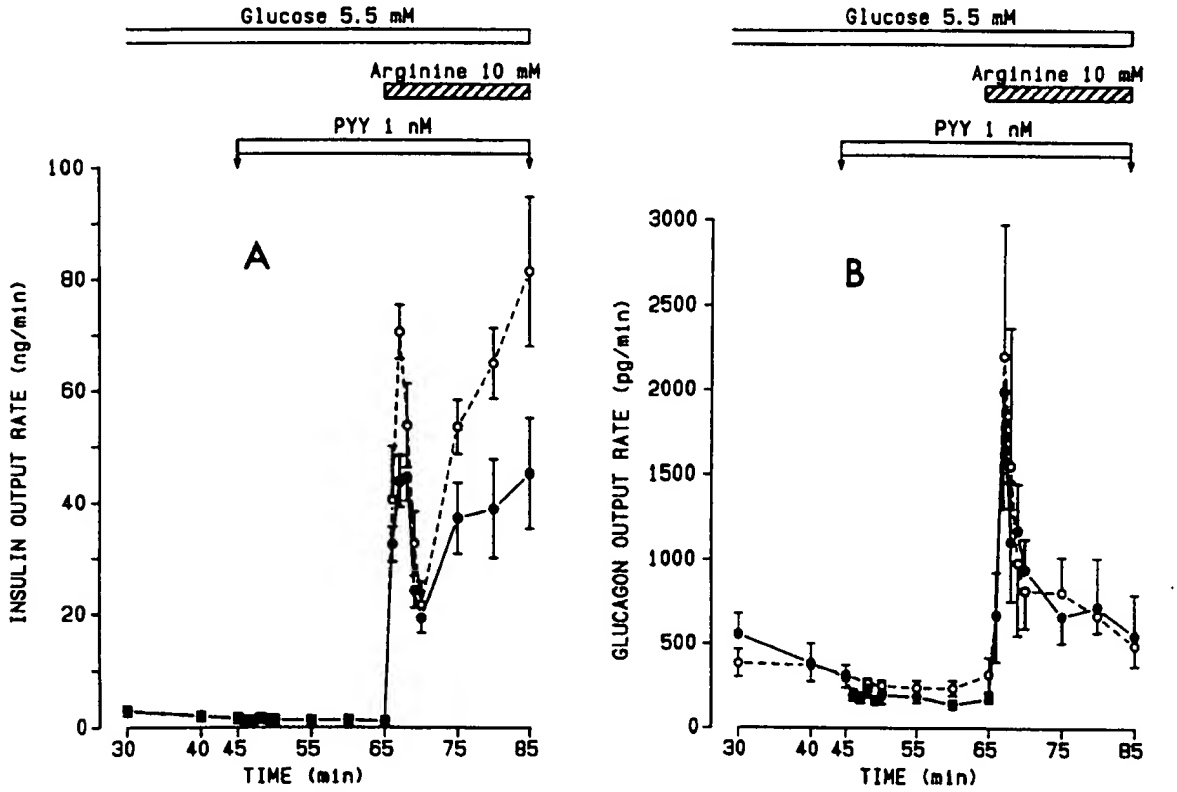

FIG. 3. Effect of PYY at 10^{-9} *M* (●) on basal and arginine (10 m*M*)-induced insulin (A) and glucagon (B) release from isolated rat pancreas perfused in the presence of 5.5 m*M* glucose. Controls (○). Values are means ± SEM of four experiments.

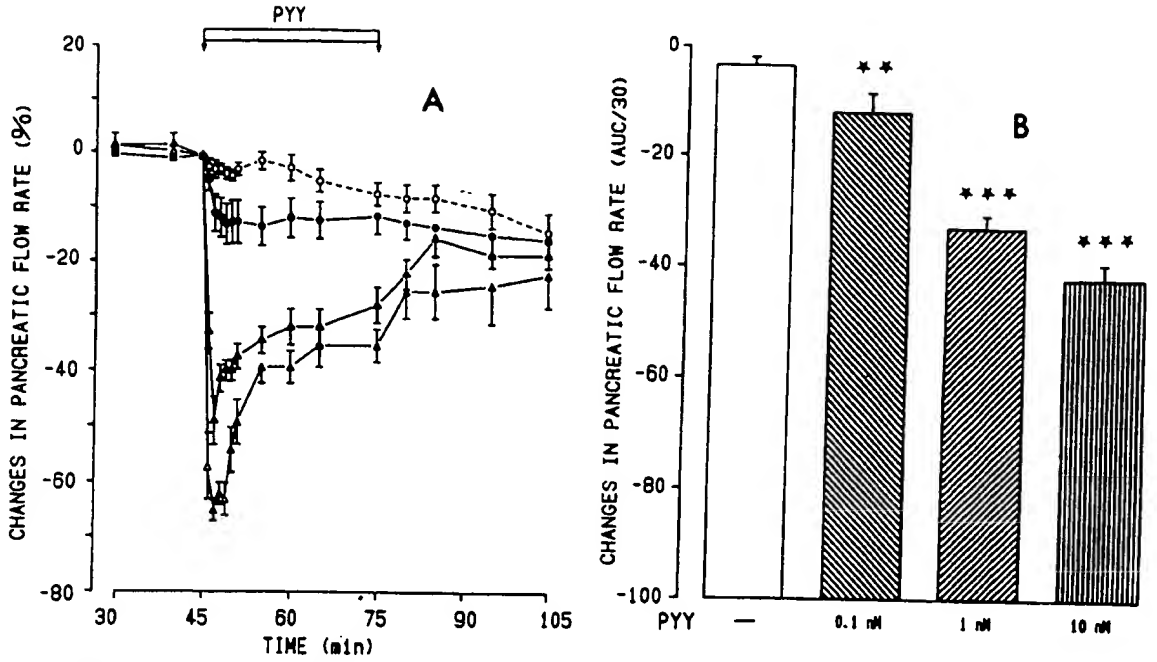

FIG. 4. Effect of PYY on pancreatic flow rate from isolated perfused rat pancreas in the presence of 8.3 m*M* glucose. A: PYY at 10^{-10} *M* (●), 10^{-9} *M* (▲), and 10^{-8} *M* (△); controls (○). B: Integrated pancreatic flow rate responses (AUC/30). The pancreatic flow rates (ml/min) at 45 min were 2.51 ± 0.02, 2.45 ± 0.05, and 2.47 ± 0.02 for PYY at 10^{-10}, 10^{-9}, and 10^{-8} *M*, respectively, and 2.49 ± 0.01 for controls. Values are means ± SEM of five experiments. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. controls.

ulation of insulin secretion, which reached 10.97 ± 0.44 ng/h/islet ($p < 0.01$). PYY at 10^{-7} *M* inhibited this glucose-induced insulin release by 27% ($p < 0.01$). The same concentration of PYY was ineffective in the presence of 3 m*M* glucose.

DISCUSSION

This study shows that PYY is able to inhibit glucose-stimulated insulin secretion and to induce a potent vasoconstriction in the isolated perfused rat pancreas. In addition, the inhibitory effect on insulin secretion was directly observed on isolated islets.

TABLE 2. *Effects of PYY on insulin release from incubated rat islets*

	Insulin secretion (ng/h/islet)
Glucose 3 m*M*	0.45 ± 0.04
Glucose 3 m*M* + PYY 10^{-7} *M*	0.44 ± 0.03
Glucose 15 m*M*	10.97 ± 0.44
Glucose 15 m*M* + PYY 10^{-7} *M*	8.03 ± 0.48

In the perfused rat pancreas, PYY (10^{-10}–10^{-9} *M*) exhibited a dose-dependent inhibitory effect on insulin secretion, with a maximum of −30%. Hence, our study suggests that the previously reported in vivo inhibition of stimulated insulin secretion by PYY (5,7) is exerted through a direct pancreatic action. The inhibitory effect of PYY on insulin secretion is comparable with that previously found with the PYY-like peptide neuropeptide Y (NPY) in perfused rat pancreas (16). However, in contrast to NPY, when the concentration of PYY was increased to 10^{-8} *M*, except during the first 2 min, the inhibitory action on insulin secretion disappeared. This unexpected result remains to be elucidated; it may suggest either a dual action on B-cells or an indirect action, i.e., via a stimulation of glucagon secretion known to stimulate B-cells (17). However, such an effect could be ruled out, since under our experimental conditions glucagon was not increased by PYY at 10^{-8} *M;* a transient reduction was even recorded. Furthermore, PYY has been previously shown to inhibit glucagon release in vivo in fed anesthetized rats (7). On the other hand, the immediate transient decrease in glu-

cagon and insulin secretions could be explained by the simultaneous drastic fall in pancreatic flow rate. Indeed, an ATP analogue, α, β-methylene ATP, despite a potent insulin stimulatory effect, induced an early and transient reduction of insulin secretion, due to a simultaneous reduction in pancreatic vascular flow rate (18,19).

PYY displayed a potent and dose-dependent vasoconstrictor effect, which occurred in a biphasic pattern at the high doses tested (10^{-9} and 10^{-8} *M*). This effect on the pancreatic vascular bed is similar to that exerted by NPY (16). Thus, PYY, like NPY, is a powerful vasoconstrictor peptide in pancreas, as previously reported in gut and submandibular salivary glands (3,20). We find that PYY reduced the vascular flow rate in a dose-dependent manner, whereas at the higher dose no sustained decrease in insulin secretion was observed. Hence, it may be concluded that the two actions of PYY in the perfused pancreas, vasoconstriction and inhibition of insulin secretion, are not causally related. Furthermore, our results on isolated islets support a direct inhibitory action of PYY on insulin secretion.

In conclusion, our study provides evidence for a direct inhibitory action of PYY on insulin secretion. As PYY is present in pancreatic endocrine cells (4,5), it might play a role in direct local inhibitory control of B-cells.

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